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PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the Application of

Erez BRAUN, Yoav EICHEN, Uri SIVAN Attn: PCT Branch

Application No. U.S. National Stage of PCT/IL99/00570

Filed: April 27, 2001 Docket No.: 109362

For: METHOD FOR GOLD DEPOSITION

SUBMISSION OF THE ANNEXES TO THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT

Director of the U.S. Patent and Trademark Office
Washington, D.C. 20231

Sir:

Attached hereto is a submission of the annexes to the International Preliminary Examination Report (Form PCT/IPEA/409). The attached submission material replaces the material in the claims.

Respectfully submitted,

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CLAIMS:

1. A method for depositing gold at one or more sites on a substrate, comprising:

(a) binding, depositing or forming nucleation centers at said one or more sites;

(b) contacting said one or more sites with a treatment composition comprising a soluble gold-providing agent, being a gold-containing molecule or complex and comprising a reagent, the composition being kinetically stable such that gold is essentially not deposited on the substrate unless a nucleation center is present on the substrate and in the presence of a nucleation center at said one or more sites, gold atoms are released from said gold-providing agent and deposited onto said nucleation center to form gold metal deposits at said one or more sites.

2. A method according to Claim 1, wherein step (a) comprises:

(a1) providing nucleation center-forming agents which comprise each at least one moiety having specific binding affinity to said one or more sites, coupled to at least one nucleation center being one or more of the group consisting of metal particle, cluster containing metal atoms and a metal-containing complex; and

(a2) contacting said one or more sites with said agents.

3. A method according to Claim 2, wherein said moiety is a member of a recognition group consisting of two or more molecules or complexes which specifically bind to one another, the other member included in or forming said one or more sites.

4. A method according to Claim 3, wherein said recognition group is a member of the group consisting of: an antigen and an antibody or an antibody derivative with an antigen-binding domain; sugar and a lectin; a receptor and a ligand; a nucleotide sequence and a complementary nucleotide sequence; a nucleotide sequence and its binding protein or other specific binding agent; biotin and avidin or streptavidin; cellulose or chitin and cellulose binding domain.

5. A method according to any one of Claims 2 to 4, wherein said nucleation center is a gold particle or a cluster containing gold atoms.

6. A method according to any one of Claims 1 to 5, wherein said treatment composition is an aqueous solution.

7. A method according to Claim 6, wherein said gold-providing agent is $Au^{I}(SCN)_2^-$.

8. A method according to Claim 7, wherein said reagent is hydroquinone or naphtohydroquinone.

9. A method for assaying presence of a specific substance at sites on a substrate, comprising:

(a) providing conditions allowing formation of nucleation centers on sites containing said substance;

(b) contacting said substrate with a treatment composition comprising a soluble providing agent, being a gold-containing molecule or complex and comprising a reagent, the composition being kinetically stable such that upon such exposure gold metal is essentially not deposited on the substrate unless a nucleation center is present thereon, and in the presence of a nucleation center at said sites, gold atoms are released from said gold-providing agent and deposited onto said nucleation center to form gold metal deposits at said sites; and

(c) detecting metallic gold deposits on said substrate, a gold deposit at a site on the substrate indicating presence of said substance at said site.

10. A method according to Claim 9, wherein step (a) comprises:

(a1) providing nucleation center-forming agents which comprise each at least one moiety, having specific binding affinity to said substance, bound to at least one nucleation center being one or more of the group consisting of metal particle, cluster containing metal atoms and a metal-containing complex; and

(a2) contacting said substrate with said agents.

11. A method according to Claim 10, wherein said moiety is included in one member of a recognition couple consisting of two molecules or complexes which

specifically bind to one another, the other member included in or constituting said substance.

12. A method according to Claim 11, wherein said recognition couple is a member of the group consisting of an antigen and an antibody or an antibody derivative with an antigen-binding domain; sugar and a lectin; a receptor and a ligand; a nucleotide sequence and a complementary nucleotide sequence; a nucleotide sequence and its binding protein or other specific binding agent; biotin and avidin or streptavidin; cellulose or chitin and cellulose binding domain; said moiety being one of the recognition couple and said substance being or including the other.

13. A method according to any one of Claims 9-12, for assaying the presence of one or more target oligonucleotides with a specific target sequence in a sample, comprising:

(a) providing a substrate carrying one or more probe oligonucleotides each with a probing sequence complementary to one of said target sequences;

(b) contacting said substrate with the sample and providing conditions allowing formation of nucleation centers where a target oligonucleotide hybridizes to a probe oligonucleotide;

(c) contacting said substrate with a treatment composition comprising a gold-providing agent, being a gold-containing molecule or complex and comprising a reagent, the composition being kinetically stable such that upon such exposure gold metal is essentially not deposited on the substrate unless a nucleation center is present thereon, and in the presence of a nucleation center at said sites, gold atoms are released from said gold-providing agent and deposited onto said nucleation center to form gold metal deposits at said sites; and

(d) detecting metallic gold deposits on said substrate, a gold deposit at a site on the substrate indicating the presence of said substance at said site.

14. A method according to Claim 13, wherein said step (b) comprises:

(b1) treating said sample to bind a label to oligonucleotides in the sample;

(b2) contacting said sample with said substrate; and

(b3) contacting said substrate with a nucleation center-containing agent, capable of specific binding to said label.

15. A method according to Claim 13, wherein said step (b) comprises:

(b1) treating said sample so as to bind nucleation centers to oligonucleotides present therein; and

(b2) contacting said samples with said substrate.

16. A method according to any one of Claims 9 to 15, wherein said nucleation center is a gold particle or a cluster containing gold atoms.

17. A method according to any one of Claims 9 to 16, wherein said treatment composition is an aqueous solution.

18. A method according to Claim 17, wherein said gold-providing agent is $Au^{I}(SCN)_2^-$ and said reagent is a quinone.

19. A method according to any one of Claims 9 to 18, wherein said substrate is a specimen for viewing in an imaging technique.

20. A method according to Claim 19, for preparing the specimen for viewing in a microscope, comprising the following steps:

(a) providing conditions permitting formation of nucleation centers at selective sites of the specimen; and

(b) contacting said specimen with a treatment composition comprising a soluble gold-providing agent, being a gold-containing molecule or complex and comprising a reagent, the composition being kinetically stable such that gold is essentially not deposited on the specimen a nucleation center is present thereon, whereby gold atoms from said gold-providing agent are released and deposited onto said specimen at selective sites.

21. A method according to any one of Claims 9 to 18, wherein said substrate contains separated fractions of a sample.

22. A method according to Claim 21 for identifying locations of a specific separation product on the substrate, comprising the steps of:

(a) providing conditions permitting formation of nucleation centers at sites on said substrate comprising said specific separation product;

(b) contacting said substrate to a treatment composition comprising a soluble gold-providing agent, being a gold-containing molecule or complex and comprising a reagent, the composition being kinetically stable such that gold is deposited on the substrate essentially only where a nucleation center is present thereon, whereby upon such contact, gold atoms from said gold-providing agent are released and deposited onto said nucleation center; and

(c) detecting presence of gold on said substrate, such gold signifying presence of said specific separation product on the substrate at a site where the gold is detected.

23. A method for assaying presence of an analyte in a sample comprising:

(a) providing a substrate carrying capturing agents which bind said analyte;

(b) contacting said substrate with said sample, whereby said analyte binds to said capturing agents;

(c) providing conditions permitting formation of nucleation centers on sites of said substrate comprising said analyte;

(d) contacting said substrate with a treatment composition comprising a soluble gold-containing molecule or complex and a reagent, the composition being kinetically stable such that gold is deposited on the substrate essentially only at sites thereof containing the nucleation centers; and

(e) detecting metallic gold deposits on said substrate, indicative of the presence of said analyte in said sample.

24. A method according to Claim 23, wherein step (c) comprises:

(c1) providing nucleation center-forming agents which comprise each at least one moiety, having specific binding affinity to said analyte, coupled to at least one nucleation center being one or more of the group consisting of metal particle, cluster of metal atoms and a metal-containing complex; and

(c2) contacting said substrate with said agents.

25. A method according to Claim 24, wherein said analyte is one of a binding couple consisting of an antigen and an antibody or an antibody derivative with an

antigen-binding domain; sugar and a lectin; a receptor and a ligand; a nucleotide sequence and a complementary nucleotide sequence; a nucleotide sequence and its binding protein or other specific binding agent; biotin and avidin or streptavidin; cellulose or chitin and cellulose binding domain; and said at least one moiety is the other of said binding couple.

26. A method according to Claim 23, wherein step (b) comprises:

(b1) contacting said sample with nucleation centers, the nucleation centers being one or more of the group consisting of metal particles, cluster of metal atoms and metal-containing complexes, and providing conditions for coupling of said nucleation centers to the analyte if present in the sample, thus obtaining a modified sample containing modified analytes; and

(b2) contacting said substrate with said modified analytes bound to said capturing agents.

27. A method according to Claim 26, wherein said analyte is one of a binding couple consisting of an antigen and an antibody or an antibody derivative with an antigen-binding domain; sugar and a lectin; a receptor and a ligand; a nucleotide sequence and a complementary nucleotide sequence; a nucleotide sequence and its binding protein or other specific binding agent; biotin and avidin or streptavidin; cellulose or chitin and cellulose binding domain; and said capturing agent is the other of said binding couple.

28. A method according to any one of Claims 23 to 27, wherein said capturing agents are carried on the substrate between electrodes such that gold deposited onto said nucleation centers in step (d) establishes an electric contact between the electrodes; and

detection of the gold deposits in step (e) is performed by measuring current-potential relationship between the electrodes.

29. A method according to any one of Claims 23 to 28, wherein said at least one metal particle, cluster of metal atoms or a metal-containing complex is a gold particle or cluster containing gold atoms.

30. A method according to any one of Claims 23 to 29, wherein said treatment composition is an aqueous solution.

31. A method according to Claim 30, wherein said gold-providing agent is $Au^{I}(SCN)_2^-$.

32. A method according to Claim 31, wherein said reagent is a hydroquinone.

33. A kit for use in the method according to any one of Claims 1 to 32.

34. A kit according to Claim 33, comprising a treatment composition which comprises a soluble gold-providing agent, being a gold-containing molecule or complex and comprises a reagent, the composition being kinetically stable such that upon contact with a substrate gold is deposited on a substrate essentially only at sites of the substrate containing nucleation centers.

35. A kit according to Claim 33 or 34, further comprising nucleation center-forming agents, for forming nucleation centers at one or more sites on the substrate, said agents comprise each at least one moiety having a specific binding affinity to a substance present at said one or more sites coupled to at least one nucleation center moiety, being one or more of the group consisting of metal particle, cluster of metal atoms and a metal containing complex.

36. A kit for use in a method according to any one of Claims 23 to 32, for assaying an analyte in a sample, comprising:

(i) a substrate carrying capturing agents which bind said analyte;

(ii) agents for forming nucleation centers at portions of the substrate on which said analyte becomes immobilized;

(iii) a treatment composition comprising a soluble gold-containing molecule or complex and a reagent, the composition being kinetically stable such that gold is deposited on the substrate essentially only at sites thereof containing the nucleation centers .

37. A kit according to Claim 36, wherein said agent for forming nucleation centers comprises a nucleation center and substances needed in order to couple said nucleation center to said analyte.

38. A kit according to Claim 36, further comprising nucleation center- forming agents which comprise each at least one moiety, having specific binding affinity to said analyte, coupled to at least one nucleation center moiety being one or more of the group consisting of metal particle, cluster of metal atoms and a metal containing complex.

39. A kit according to any one of Claims 36 to 38, wherein said substrate comprises two or more electrodes, and said capturing agents are carried within gaps between the electrodes.

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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

14

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
119277.2 MM		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/IL99/00570	27/10/1999	27/10/1998
International Patent Classification (IPC) or national classification and IPC G01N33/543		
Applicant TECHNION RESEARCH AND DEVELOPMENT FOUN... et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 8 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 9 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☒ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
27/04/2000	20.02.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Luis Alves, D Telephone No. +49 89 2399 8695

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/IL99/00570

I. Basis of the report

1. This report has been drawn on the basis of *(substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments (Rules 70.16 and 70.17).)*:
Description, pages:

1-18	as originally filed

Claims, No.:

1-41	as received on	16/11/2000	with letter of	14/11/2000

Drawings, sheets:

1/6-6/6	as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
   ☐ the language of publication of the international application (under Rule 48.3(b)).
   ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   ☐ contained in the international application in written form.
   ☐ filed together with the international application in computer readable form.
   ☐ furnished subsequently to this Authority in written form.
   ☐ furnished subsequently to this Authority in computer readable form.
   ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
   ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:
   ☐ the claims, Nos.:

☐ the drawings, sheets:

5. ☒ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)
see separate sheet

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-20, 22-41
	No:	Claims	
Inventive step (IS)	Yes:	Claims	7, 8, 18, 33
	No:	Claims	1-6, 9-17, 19, 20, 22-32, 34-41
Industrial applicability (IA)	Yes:	Claims	1-20, 22-41
	No:	Claims	

2. Citations and explanations
see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

Reference is made to the following documents cited in the International search report:

D1: US-A-5 202 151
D2: US-A-5 284 748

Section I:

Point 5.

The subject-matter of new claim 21 does not comply with the requirements of Article 34 PCT because no basis could be found in the originally filed application for a detection technique based on a combination of absorbance, transmission, reflectance and scattering, as defined by the use of "and/or" in claim 21.
Consequently, this report is established disregarding said unallowable amendment, and accordingly no opinion is formulated on the subject-matter of claim 21.

Section V:

The present report is based on amended claims 1 to 20 and 22 to 41, as indicated under Section I of the report. However, the report is established as if the following amendments to claim 1, for which no basis could be found in the originally filed application, had not been made: The deletion of the term "specifically" in step (a); The introduction of the term "molecules" at the end of step (a), which term could not be found in the originally filed application in connection with the step of providing nucleation centers. For the same reason, the term "molecules" at the end of claims 3, 4, 16, 28 and 37 has been disregarded. The expression "and metals" at the end of new claim 2 has also been disregarded because no basis could be found for said expression in the originally filed application.

1. D1 discloses a solution for deposition of gold which comprises a metal complex and a reducing agent such as hydroquinone, and which is applied to a substrate after a gold coating has been applied (see example 1). The gold coating acts as nucleation centers for the treatment composition applied subsequently, even if in D1 said gold coating is not specifically described as the formation of nucleation centers. D1 discloses treatment compositions which result in gold deposition only on the desired surfaces and which are described as stable compositions. Thus, the compositions disclosed in D1 fulfill the stability requirements defined in present claim 1(b).
D1 does not disclose kits. However, the subject-matter of claims 35 and 36 does not seem to involve an inventive step over D1 because the incorporation into a kit of known compounds for the performance of a known method (the method disclosed in D1) is obvious (Article 33(3) PCT).

2. D2, which is considered to represent the closest prior art with respect to independent claims 1, 9 and 25, discloses methods which are distinguished from said claimed methods only in that the development step of the method consists of silver deposition rather than gold deposition. Although D2 also refers to the possibility of performing said development step using a different metal, such as gold (see column 4 to column 6, column 11, lines 39 to 62 and Figure 11), the deposition of gold is not specifically described.

Although D2 concerns coating of the nucleation centers and requires presence of the binding partner to the analyte over the whole space bridging the electrodes, present claims 1, 9 and 25 also comprise such an embodiment and therefore this feature does not constitute a distinction between said claims and D2.

The expression "under appropriate conditions" in present claim 1, and the implicit requirement for appropriate conditions in the methods in claims 10 (which also comprises the binding between two members of a binding pair) and independent claim 25, are merely statements of a problem to be solved, since the relevant features allowing to perform the methods at said appropriate conditions are not defined.

Thus, the problem to be solved by present claims 1, 9 and 25 with respect to D2 may be seen as the provision of a method in which the developing step is performed using a metal other than silver. The solution, which consists of the replacement of silver deposition by gold deposition, appears to be obvious from D2 in combination with D1 because the use of gold deposition is already suggested in D2 and the skilled person would known from D1 how to perform it. Thus, the subject-matter of claims 1, 9 and 25 does not seem to involve an inventive step (Article 33(3) PCT).

All the features in dependent claims 10 to 16 and 26 to 30 are also disclosed in D2: With respect to claims 10 to 12, 16 and 26 to 30 see column 4, lines 21 to 36 and claim 1; with respect to claims 13 to 15 see claims 1 and 10.
Further, the uses defined in dependent claims 19, 20 and 22 to 25 appear to be obvious and not to involve an inventive step in view of D2 in combination with D1.
Therefore, the subject-matter of dependent claims 10 to 16, 19, 20, 22 to 25 and 26 to 30 does not comply with the requirements of Article 33(3) PCT.
The characteristics of the treatment composition for deposition of gold, which are the object of claims 6, 17, 32 and 34 are already known from D1, as discussed above. Thus, they do not render inventive the subject-matter of said claims (Article 33(3) PCT).
Since the methods defined in independent claims 9 and 25 are considered to lack an inventive step, no inventive activity can be seen in providing kits for use in said methods. Therefore, the subject-matter of claims 37 to 41 does not comply with the requirements of Article 33(3) PCT.
Dependent claims 2 to 5 concern features disclosed in D2, as discussed above. Thus, the subject-matter of claims 2 to 5 does not comply with the requirements of Article 33(3) PCT.

3. On the other hand, the use in the methods of the specific reagents defined in claims 7, 18 and 33 is not suggested in any of the available documents and furthermore allows to perform the methods at more favourable conditions. Said claims comply with the requirements of Article 33(2) and (3) PCT. Claim 8 refers

back to claim 7 and therefore also complies with the requirements of Article 33(2) and (3) PCT.

Section VII:

1. Contrary to the requirements of Rule 5.1(a)(ii) PCT, the relevant background art disclosed in documents D1 and D2 is not mentioned in the description, nor are these documents identified therein.

Section VIII:

1. The subject-matter of claim 35 is not defined because the components of the kit are not described. Thus, said claim does not comply with the requirements of Article 6 PCT.

2. The term "substances needed in order to" used in claim 39 is vague and renders the definition of the subject-matter of said claim unclear (Article 6 PCT). The components required should be defined in the claim.

4. The method steps defined in claim 23, which refers back to independent claim 9, are already comprised in the method defined in claim 9. This repetition of steps renders unclear the subject-matter of both said claims (Article 6 PCT). As formulated, claim 23 appears to be directed to a method consisting of the same steps as in claim 9, but wherein the substrate is a specimen for viewing in a microscope. Claim 23 should clearly identify the additional steps distinguishing it from claim 9.
   The same objection applies to dependent claim 25 (Article 6 PCT).

5. Step (a) of amended claim 1 is defined in terms of the result (nucleation centers) achieved in the method step. Since claim 1 concerns a method, it would be more appropriately defined in terms of the method steps (Article 6 PCT). Thus, the subject-matter of original claim 2, which has been introduced into present claim 1, should be introduced as in the original claim 2 by definition of method steps:

(a2) "contacting said one or more sites with nucleation center forming agents" and (a2) "each of said agents comprising...".

6. The subject-matter of claim 4 is already defined in claim 3. Thus, the claims lack conciseness (Article 6 PCT).

7. Dependent claim 25 should refer back to claim 24, not to claim 23.

8. There are two claims numbered 25. The claims following the first claim 25 should be renumbered 26 to 42.

9. The description has not been adapted to the amended claims.

CLAIMS:

1. A method for depositing gold at one or more sites on a substrate, comprising:

(a) binding, depositing or forming nucleation centers at said one or more sites; said nucleation centers comprising at least one member of a recognition group consisting of two or more {}molecules or complexes which bind {}to each other, the other member including or forming said one or more sites, coupled to at least one nucleation center being one or more of the group consisting of metal particle, cluster containing metal atoms, a metal-containing complex and molecules.

(b) Contacting under appropriate conditions said one or more sites with a treatment composition comprising a soluble gold-providing agent, being a gold-containing molecule or complex and comprising a reagent, the composition being kinetically stable such that gold is essentially not deposited on the substrate unless a nucleation center is present on the substrate and in the presence of a nucleation center at said one or more sites, gold atoms are released from said gold-providing agent and deposited onto said nucleation center to form gold metal deposits at said one or more sites.:

2. A method according to Claim 1, wherein the one member of the recognition group is coupled to at last one nucleation center, being one or more of the group consisting of: cluster containing metal atoms and metal containing complexes and metals.

3. A method according to Claim 1, wherein the one member of the recognition group is coupled to at least one nucleation center being one or more of the group consisting of gold particle, cluster containing gold atoms and gold-containing complexes and molecules.

4. A method according to Claim 1, wherein the one member of the recognition group is coupled to at least one nucleation center being one or more of the group consisting of cluster containing gold atoms and gold-containing complexes and molecules.

5. A method according to Claim 1, wherein said {} recognition group is a member of the group consisting of: an antigen and an antibody or an antibody derivative with an antigen-binding domain; sugar and a lectin; a receptor and a ligand; a nucleotide sequence and a complementary nucleotide sequence; a nucleotide sequence and its binding protein or other specific binding agent; biotin and avidin or streptavidin; cellulose or chitin and cellulose binding domain.

6. A method according to any one of Claims 1 to 5, wherein said treatment composition is an aqueous solution.

7. A method according to Claim 6, wherein said gold-providing agent is $Au^{I}(SCN)_2^-$.

8. A method according to Claim 7, wherein said reagent is hydroquinone or naphtohydroquinone.

9. A method for assaying presence of a specific substance at sites on a substrate, comprising:

(a) providing conditions allowing formation of nucleation centers on sites containing said substance;

(b) contacting said substrate with a treatment composition comprising a soluble gold providing agent, being a gold-containing molecule or complex and comprising a reagent, the composition being kinetically stable such that upon such exposure gold metal is essentially not deposited on the substrate unless a nucleation center is present thereon, and in the presence of a nucleation center at said sites, gold atoms are released from said gold-providing agent and deposited onto said nucleation center to form gold metal deposits at said sites; and

(c) detecting metallic gold deposits on said substrate, a gold deposit at a site on the substrate indicating presence of said substance at said site.

10. A method according to Claim 9, wherein step (a) comprises:

(a1) providing nucleation center-forming agents which comprise each at least one moiety, having specific binding affinity to said substance, bound to at least one nucleation center being one or more of the group consisting of metal particle, cluster containing metal atoms and a metal-containing complex; and

(a2) contacting said substrate with said agents.

11. A method according to Claim 10, wherein said moiety is included in one member of a recognition couple consisting of two molecules or complexes which specifically bind to one another, the other member included in or constituting said substance.

12. A method according to Claim 11, wherein said recognition couple is a member of the group consisting of an antigen and an antibody or an antibody derivative with an antigen-binding domain; sugar and a lectin; a receptor and a ligand; a nucleotide sequence and a complementary nucleotide sequence; a nucleotide sequence and its binding protein or other specific binding agent; biotin and avidin or streptavidin; cellulose or chitin and cellulose binding domain; said moiety being one of the recognition couple and said substance being or including the other.

13. A method according to any one of Claims 9-12, for assaying the presence of one or more target oligonucleotides with a specific target sequence in a sample, comprising:

(a) providing a substrate carrying one or more probe oligonucleotides each with a probing sequence complementary to one of said target sequences;

(b) contacting said substrate with the sample and providing conditions allowing formation of nucleation centers where a target oligonucleotide hybridizes to a probe oligonucleotide;

(c) contacting said substrate with a treatment composition comprising a gold-providing agent, being a gold-containing molecule or complex and comprising a reagent, the composition being kinetically stable such that upon such exposure gold metal is essentially not deposited on the substrate unless a nucleation center is present thereon, and in the presence of a nucleation center at said sites, gold atoms are released from said gold-providing agent and deposited onto said nucleation center to form gold metal deposits at said sites; and

(d) detecting metallic gold deposits on said substrate, a gold deposit at a site on the substrate indicating the presence of said substance at said site.

14. A method according to Claim 13, wherein said step (b) comprises:

(b1) treating said sample to bind a label to oligonucleotides in the sample;

(b2) contacting said sample with said substrate; and

(b3) contacting said substrate with a nucleation center-containing agent, capable of specific binding to said label.

15. A method according to Claim 13, wherein said step (b) comprises:

(b1) treating said sample so as to bind nucleation centers to oligonucleotides present therein; and

(b2) contacting said samples with said substrate.

16. A method according to any one of Claims 9 to 15, wherein said nucleation center is a gold particle a cluster containing gold atoms, or a gold-containing complex or molecules.

17. A method according to any one of Claims 9 to 16, wherein said treatment composition is an aqueous solution.

18. A method according to Claim 17, wherein said gold-providing agent is $Au^{I}(SCN)_2^-$ and said reagent is a quinone.

19. A method according to any one of Claims 9 to 18, wherein said substrate is a specimen for viewing in an imaging or visualization technique.

20. A method according to Claim 19, wherein the detection technique is based on light.

21. A method according to Claim 19, wherein the {} detection technique is based on light absorbance and/or light transmission and/or light reflectance and/or light scattering.

22. A method according to Claim 19, wherein said detection is maid by techniques selected rom: tansmission electron microscopy, scanning electron microscopy, atomic force microscopy.

23. A method according to Claim 19, for preparing the specimen for viewing in a microscope, comprising the following steps:

(a) providing conditions permitting formation of nucleation centers at selective sites of the specimen; and

(b) contacting said specimen with a treatment composition comprising a soluble gold-providing agent, being a gold-containing molecule or complex and comprising a reagent, the composition being kinetically stable such that gold is essentially not deposited on the specimen a nucleation center is present thereon, whereby gold atoms from said gold-providing agent are released and deposited onto said specimen at selective sites.

24. A method according to any one of Claims 9 to 18, wherein said substrate contains separated fractions of a sample.

25. A method according to Claim 23 for identifying locations of a specific separation product on the substrate, comprising the steps of:

(a) providing conditions permitting formation of nucleation centers at sites on said substrate comprising said specific separation product;

(b) contacting said substrate to a treatment composition comprising a soluble gold-providing agent, being a gold-containing molecule or complex and

comprising a reagent, the composition being kinetically stable such that gold is deposited on the substrate essentially only where a nucleation center is present thereon, whereby upon such contact, gold atoms from said gold-providing agent are released and deposited onto said nucleation center; and

(b) detecting presence of gold on said substrate, such gold signifying presence of said specific separation product on the substrate at a site where the gold is detected.

25. A method for assaying presence of an analyte in a sample comprising:

(a) providing a substrate carrying capturing agents which bind said analyte;

(b) contacting said substrate with said sample, whereby said analyte binds to said capturing agents;

(c) providing conditions permitting formation of nucleation centers on sites of said substrate comprising said analyte;

(d) contacting said substrate with a treatment composition comprising a soluble gold-containing molecule or complex and a reagent, the composition being kinetically stable such that gold is deposited on the substrate essentially only at sites thereof containing the nucleation centers; and

(e) detecting metallic gold deposits on said substrate, indicative of the presence of said analyte in said sample.

26. A method according to Claim 25, wherein step (c) comprises:

(c1) providing nucleation center-forming agents which comprise each at least one moiety, having specific binding affinity to said analyte, coupled to at least one nucleation center being one or more of the group consisting of metal particle, cluster of metal atoms and a metal-containing complex; and

(c2) contacting said substrate with said agents.

27. A method according to Claim 26, wherein said analyte is one of a binding couple consisting of an antigen and an antibody or an antibody derivative with an antigen-binding domain; sugar and a lectin; a receptor and a ligand; a

nucleotide sequence and a complementary nucleotide sequence; a nucleotide sequence and its binding protein or other specific binding agent; biotin and avidin or streptavidin; cellulose or chitin and cellulose binding domain; and said at least one moiety is the other of said binding couple.

28. A method according to Claim 25 wherein step (b) comprises:

(b1) contacting said sample with nucleation centers, the nucleation centers being one or more of the group consisting of metal particles, cluster of metal atoms and metal-containing complexes or molecules, and providing conditions for coupling of said nucleation centers to the analyte if present in the sample, thus obtaining a modified sample containing modified analytes; and

(b2) contacting said substrate with said modified analytes bound to said nucleation centers.

29. A method according to Claim 28, wherein said analyte is one of a binding couple consisting of an antigen and an antibody or an antibody derivative with an antigen-binding domain; sugar and a lectin; a receptor and a ligand; a nucleotide sequence and a complementary nucleotide sequence; a nucleotide sequence and its binding protein or other specific binding agent; biotin and avidin or streptavidin; cellulose or chitin and cellulose binding domain; and said capturing agent is the other of said binding couple.

30. A method according to any one of Claims 25to 29, wherein said capturing agents are carried on the substrate between electrodes such that gold deposited onto said nucleation centers in step (d) establishes an electric contact between the electrodes; and

detection of the gold deposits in step (e) is performed by measuring current-potential relationship between the electrodes.

30. A method according to any one of Claims 25to 30, wherein said at least one metal particle, cluster of metal atoms or a metal-containing complex is a gold particle or cluster containing gold atoms.

32. A method according to any one of Claims 25 to 31, wherein said treatment composition is an aqueous solution.

33. A method according to Claim 32, wherein said gold-providing agent is $Au^{I}(SCN)_2^-$. 34 A method according to Claim 32, wherein said reagent is a hydroquinone.

35. A kit for use in the method according to any one of Claims 1 to 34

36. A kit according to Claim 35, comprising a treatment composition which comprises a soluble gold-providing agent, being a gold-containing molecule or complex and comprises a reagent, the composition being kinetically stable such that upon contact with a substrate gold is deposited on a substrate essentially only at sites of the substrate containing nucleation centers.

37. A kit according to Claim 35 or 36, further comprising nucleation center-forming agents, for forming nucleation centers at one or more sites on the substrate, said agents comprise each at least one {} member of a recognition group having a specific binding affinity to a substance present at said one or more sites coupled to at least one nucleation center moiety, being one or more of the group consisting of metal particle, cluster of metal atoms and a metal containing complex or molecule.

38. A kit for use in a method according to any one of Claims 25t o 34, for assaying an analyte in a sample, comprising:

(i) a substrate carrying capturing agents which bind said analyte;

(ii) agents for forming nucleation centers at portions of the substrate on which said analyte becomes immobilized;

(iii) a treatment composition comprising a soluble gold-containing molecule or complex and a reagent, the composition being kinetically stable such that gold is deposited on the substrate essentially only at sites thereof containing the nucleation centers .

39. A kit according to Claim 38, wherein said agent for forming nucleation centers comprises a nucleation center and substances needed in order to couple said nucleation center to said analyte.

40. A kit according to Claim 38, further comprising nucleation center-forming agents which comprise each at least one moiety, having specific binding affinity to said analyte, coupled to at least one nucleation center moiety being one or more of the group consisting of metal particle, cluster of metal atoms and a metal containing complex.

41. A kit according to any one of Claims 38 to 40, wherein said substrate comprises two or more electrodes, and said capturing agents are carried within gaps between the electrodes.